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Review

Towards an artificial cell

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ABSTRACT

We are on the verge of producing "synthetic cells," or protocells, in which some, many or all of the tasks of a real biological cell are harnessed into a synthetic platform. Such advances are made possible through genetic engineering, microfabrication technologies, and the development of cellular membranes from new surfactants that extend beyond phospholipids in stability and chemical control, and can be used to introduce designer functionality into membranes and cells. We review some of the recent advances in the development of synthetic cells and suggest future exciting directions.

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1. Introduction

We are at the verge of a revolution in biology, where we can reconstruct some of life's functions within synthetic materials. Synthetic biology, a new and rapidly evolving subfield of biotechnology, endeavors to embed enhanced functionality and response within existing biological cells, usually using recombinant biotechnology to include genetic circuits that are not normally present. In doing so, we learn the limits of signaling responses in biological cells, develop new dynamic control mechanisms and motifs, and develop greater insight into the fundamental elements that control cell behavior.

Starting from the ground up, we can construct cellular mimetic structures that are inspired by and made in the likeness of a natural cell. An artificial cell, sometimes referred to as a protocell, is an ordered structure, enclosed by a membrane, that carries out some life activities, such as signaling, communication, sensation or growth [4]. Rasmussen and co-workers have discussed the importance of understanding the transition that occurs from non-living materials and chemistry to the construction of a living system and our current concept of biology. These authors have stated that attempts to build a living, protocell structure need not be limited by using purely biotic components or materials found or used in current biological cells. Rather, our goal as scientists in this emerging field of constructing artificial cells should be to create systems that demonstrate the essential features we associate with being 'alive'. These essential functions include (1) localization of components, (2) growth, maintenance and self-replication, and (3) the potential for evolution through the replication and transfer of internal components or genetic information [5].

We observe that approaches to create these types of synthetic systems vary and contain a further hierarchal division into top-down or bottom-up techniques. For example, some researchers have recreated these essential cellular features through controlled assembly of very basic chemical building blocks, while others aim to mimic the higher level functions and features that are derived from these basic processes, like autonomous movement, dynamic and responsive membranes, or compartmentalization. While the former approach would allow us to understand the transition from chemistry to biology that accompanied the development of living cells, the latter approach allows us to understand thermodynamic principles involved in higher order cell functions and develop a tool kit of cellular behaviors that we can assemble to create intelligent devices for future applications in medicine and biotechnology.

With either approach, we begin with a vesicle membrane as a scaffold upon which we can build higher order complex behaviors that are normally generated by genetic circuits in cells. Building an artificial cell involves the orderly assembly of multiple elements within or encapsulated by a synthetic membrane. Ultimately, insights from synthetic biology may be used to embed designed circuitry within synthetic capsules. Over time, the concepts from synthetic biology and the engineering of artificial cells may merge, where synthetic control motifs designed by recombinant biotechnology are incorporated within synthetic shells.

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The goal of this brief review is to summarize recent accomplishments in the engineering of artificial cells, or protocells, and establish new challenges in this emerging but exciting discipline.

2. Genetic engineering and synthetic biology

Synthetic biology emerged as a discipline in the last decade as scientists attempted to use the tools of molecular biotechnology to design signaling circuits with precise temporal response into biological cells. In a pair of papers appearing in Nature in 2000, two distinct laboratories reported the design of a toggle switch [6], with the binary response of a digital circuit, and an oscillator achieved by the sequential repression of adjacent genes on a single plasmid (repressilator) [7].

An incomplete list of signaling motifs that can be incorporated within cells include communication systems that respond optically to different concentrations of small analytes, similar to a band gap filter [8], the design of on–off protein motifs with various sensitivities to concentration [9], and enhanced redesign of signaling cascades to increase or modulate the ultra-sensitivity of cellular responses [10]. The design of circuitry is not limited to the design of proteins. For example, RNA switches (aptamers) can be used to control biological control processes, such as the reading (or the inhibition of reading) of messenger sequences in response to the presences of small analytes [11]. Also, RNA control and protein control elements can be combined to create exquisitely sensitive intracellular control systems [12].

Substantial work on modeling classical regulatory motifs has identified modules that can give rise to virtually any response, including oscillatory responses or feedback control motifs in which signals are persistently on or off once the input has passed a critical threshold [13]. Since biology routinely employs complex machines to make simple decisions, knowledge about the coupling between intracellular signaling and phenotypic responses may ultimately be very useful in designing decision loops in protocells using minimal components.

A most significant achievement in combining synthetic biology circuitry and construction of a synthetic cell was the redesign of the complete genome of a simple living organism, achieved recently by Venter and co-workers [14]. A very large DNA strand was created synthetically through ab initio design and splicing small sequences of DNA into a large strand that was able to contain the complete instruction set for the replication and maintenance of cellular activity. This approach defines the requirements for the full maintenance of biological processes; such insights can ultimately be used to design full instruction sets within synthetic cells.

3. New membranes

Natural biological membranes are made from bilayers of diacyl chain phospholipids with various other surfactant components. The acyl chains of these lipids typically contain between 14 and 20 carbons and can have different degrees of unsaturation. Additional components in biological membranes are steroids and steroidal-like surfactants, such as cholesterol. Cells obviously possess a synthetic machinery to synthesize these lipid components.

A theme of our laboratory and others has been to extend the palette of molecules that can assemble into membranes, and thus make synthetic membranes with designed properties. With wider synthetic control of the membrane, it would be possible to tune the materials strength and catalytic activity, and to envisage properties that are not readily achievable through lipids. As suggested above, the family of phospholipids is rather narrow, and as a result, membrane properties of synthetic lipid vesicles are rather narrowly prescribed. For example, it is known that phospholipid lipid membranes are resistant to changes in area, and fail upon stretching to just several areal percent [15]. Thus, one would expect membranes constructed from different surfactants to display different physical and chemical properties.

A leap forward involved the assembly of membranes from amphiphilic block co-polymers [16]. Vesicles surrounded by block co-polymer membranes are called polymersomes. Over the past decade or so, numerous polymers of different chemistry have been shown to assemble into membranes [4,17]. Because the polymers have a larger molecular weight than lipids and the polymers can be somewhat entangled, the membranes can store considerable elastic energy through changes in area [18]. Furthermore, the addition of side chains to the polymer can be used to crosslink the membrane, making a hyper-tough shell reminiscent of the rheological properties of a cellular membrane composite [19].

Because the membranes are often hyperthick compared to lipid membranes, larger solutes can be incorporated within the vesicle membrane, such as hydrophobic porphyrins that allow vesicles to emit light [1,20–24]. These embedded porphyrinic solutes have allowed us to construct light-sensitive membranes that fall apart in response to illumination [23,24], and optical stress sensors that allow us to correlate the degree of membrane deformation with changes in the wavelength of optical emission [1] (see illustration, Fig. 1).

Another emerging area of interest is the creation of synthetic membranes from bio-inspired building blocks. Since polypeptides are derived entirely from natural materials, their continued development into membrane-forming structures promises to be an exciting addition to the current library of materials for assembling vesicles. The first example of protein materials assembling into vesicles was reported by Deming and co-workers [25]. Bridging the gap between block copolymers and proteins, they synthesized a series of block co-polypeptides of poly(leucine) and poly(lysine, arginine, or aspartic acid) that readily assemble into vesicles [25–27]. Membrane formation is driven by hydrophobic collapse of leucine α-helices and stabilization by the highly charged lysine, arginine, or aspartic acid block. Interestingly, membranes of block-co-peptides can be assembled with amphiphiles that contain much larger hydrophilic block fractions than synthetic polymers [28]. Addition of non-canonical amino acids, such as dihydroxyphenylalanine (DOPA), was achieved by mixing DOPA with other amino acids during co-polymerization [29]. One limitation with the co-polymerization of amino acids into blocks, however, is that the precise sequence of amino acids down to the single amino acid unit cannot be controlled. While the mean local concentration of amino acids and the mean chain length of the peptide can be regulated, the precise sequence of residues – so often important for the control of biological processes – cannot be regulated reliably.

Li and co-workers [30] created a polymer vesicle where a triblock co-peptide could be incorporated in a synthetic membrane at up to 70% membrane loading. They assembled block co-polypeptides into Pluronic L121 (PEO-poly(propylene oxide)) vesicles. These peptides were obtained through yeast expression and could be triggered to incorporate into a membrane in response to pH changes.

A natural next step in the evolution of protocells is the construction of membranes from proteins made by recombinant biotechnology. Recombinantly made proteins would offer advantages over the systems mentioned above, because they would allow the assembly of membranes from monodisperse macromolecules made from naturally occurring biological building blocks (amino acids). Furthermore, functionalization – to include peptides or other functional motifs, such as enzymes or protease cleavable domains – would be trivially achieved by modifying the gene of interest before insertion in the vector.

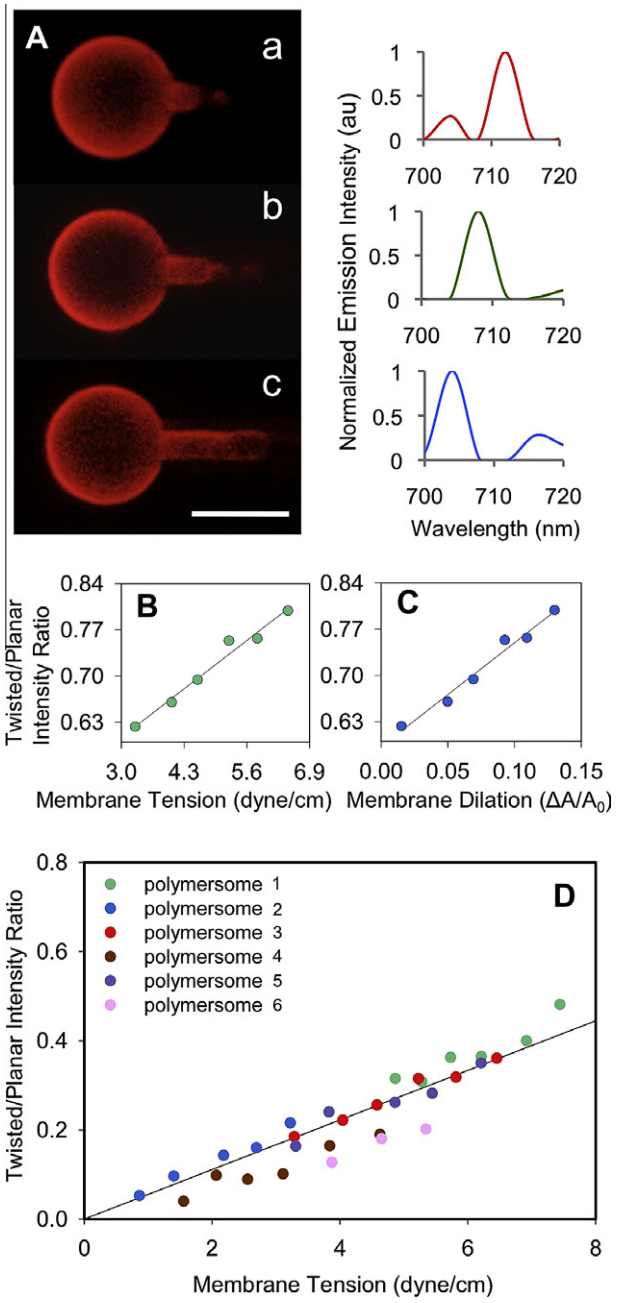

Fig. 1. A stress sensor. (A) Aspiration of a polymersome membrane into a micropipette results in a blue shift of emission of a porphyrin-based fluorophore, PZn_2, in response to the applied membrane stress. Increasing the tension in the polymersome membrane from (a) 0.8 dyne/cm to (b) 1.7 dyne/cm to (c) 4.7 dyne/cm, causes porphyrin fluorophores to emit at lower wavelengths. Scale bar is 30 μm (B) The fraction of PZn_2 fluorophores in a twisted state linearly increases with increasing membrane tension. The ratio of emission intensities at $\lambda_{twisted}$ = 690 nm to λ_{planar} = 730 nm was determined using a PMT and 730 ± 10 nm and 690 ± 10 nm band pass emission filters. (C) Tension causes an increase in area expansion and results in a porphyrin blue shift. The increase in membrane area scales linearly with the increase of fluorophores in the twisted state. The 690 nm/730 nm intensity ratios for several polymersomes undergoing aspiration shows an increase in PZn_2 intensity ratio with applied tension. The average slope, calculated from the best-fit line of each polymersome, is displayed on the chart. Figure used with permission from Ref. [1].

The Hammer laboratory recently achieved the objective of making vesicles from a recombinant protein [2] (see Fig. 2). Starting with oleosin, a well-known plant protein that acts as a surfactant to solubilize fat, we have made a family of modified molecules that assemble into a variety of molecular suprastructures in solution, including vesicles, fibers, and sheets [2]. Oleosin is structured with three sections – a central hydrophobic section flanked by two hydrophilic arms. In the center of the hydrophobic core there is a proline knot that forces a turn, so that the molecule appears as a "U" in solution, and acts as a linear surfactant.

Starting with the wild-type molecules, a family of truncation mutants was made. A minor truncation to the hydrophobic core involved reduction of the number of amino acids from 87 to 65 (11 residues removed from each arm). Then, a series of truncations were performed on the hydrophilic arms. A broad class of molecules assemble into vesicles, as was verified by cryo-transmission electron microscopy (cryo-TEM). A variety of other truncations form fibers and sheets. There is a generally a complex interplay between protein structure, pH, and solution ionic strength that modulated the formation of vesicular structures. For example, for proteins that form vesicles in solution in physiological media (in which the ionic strength was high), reduction of the ionic strength induced repulsion between the head groups of the surfactant, leading to induced curvature and the formation of fibers. This work included a systematic mapping of the phase diagram of the relationship between protein size, solvent condition, and the resulting structures of protein assemblies in solution that can provide a roadmap for future systematic development of recombinant proteins into macromolecular superstructures.

Considerable work has also been done with elastin-like polypeptides, which have also been produced and purified using recombinant methods [31]. These peptides have been used to make micelles and for tissue engineering applications. Elastin-like polypeptides can be engineered to possess a temperature switch that allows disassembly and reassembly of the peptide micelle [32]. Attempts have been made to assemble vesicles from recombinantly-produced elastin peptides, but despite alluring evidence from dynamic light scattering of the formation of structures consistent with vesicle formation, evidence from cryo-TEM was lacking [33]. Further research and characterization of these structures will be needed to verify that they indeed form vesicles.

Another type of surfactant that has recently received considerable attention is peptide amphiphiles [34,35]. Generally, peptide amphiphiles involve the annealing of a short peptide (3–15 amino acids in length) to an acyl chain surfactant. A closely related approach is to make peptides entirely by solid phase synthesis [36]. Despite the many uses of these materials for making micelles or sheets for tissue engineering, to our knowledge, these materials have not been assembled into membranes or vesicles as of yet.

Making membranes from proteins holds an obvious advantage for the development of protocells. By incorporating cell free recombinant machinery into the interior of a vesicle, the production of a surfactant protein can lead to the regeneration of the membrane itself. In fact, incorporation of multiple motifs might lead to continuous regeneration of the properties of the membrane, leading to capsule function evolving with time.

4. Protocells

We now summarize some of the behaviors of protocells that can be incorporated within synthetic capsules.

4.1. Catalysis

The presence of a metabolic system is an essential goal of constructing a protocell and has long defined 'living' organisms. The signaling cascades and feedback loops that facilitate the autonomous and intelligent behavior of a cell are regulated through a series of intracellular chemical reactions. These reaction networks

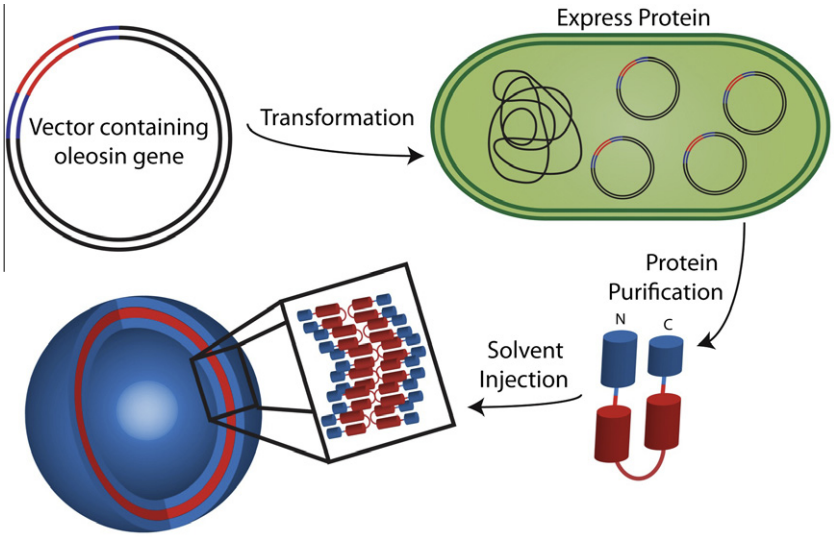

Fig. 2. Vesicles were created from a recombinant protein, oleosin. The gene for oleosin was inserted into a vector and added to bacteria. Numerous oleosin variants were expressed, many of which formed vesicles, but some which formed sheets and fibers. Figure taken with permission from Ref. [2].

continuously regulate one another in order to maintain chemical equilibrium and enable physical changes in cell structure that promote processes like spreading, motility and division. By compartmentalizing metabolic processes, a cell can maintain multiple distinct environments that enable the precise control of chemical synthesis and intracellular communication.

A simple method to mimic an intracellular enzymatic reaction is to contain a single enzyme reaction in a single compartment. Meier and co-workers [37] demonstrated this by incorporating a channel-forming protein, OmpF, into the membrane of a polymersome. An internally encapsulated β-lactamase hydrolyzed substrates that permeated the vesicle membrane. Since then, reconstituted enzymatic reactions housed within the polymer membranes have advanced to the stage of multiple enzyme systems. Several recent reviews have recently summarized advances in the field of synthetically-based nanoreactors [38,39].

In order to replicate the signaling cascades that occur within cells, metabolic processes will need to become coupled. Communication of signaling systems has been engineered both through capsule to capsule signaling and through signaling within a single synthetic compartment. As an example of the former, an enzymatically-active polymersome was made to communicate with another polymersome, creating a signaling cascade between two individual compartments [40]. As an example of the latter, multiple enzymes were partitioned into different locations of a polymersome and a reaction cascade was created between them. Finally, the complexity of the enzyme-containing polymersome can be enhanced by increasing the number of enzymes in a single polymersome [40]. By localizing three separate enzymes to the membrane surface, membrane bilayer core, and aqueous lumen of polymersomes, respectively, the van Hest group enabled site-specific positioning of enzymes in a single vesicle [41]. These enzymes created a cascade of reactions and set the stage for creating colonies of communicating polymersomes, each containing multiple enzymes and signaling cascades in their interior.

Metabolic processes that generate energy are essential for developing of a self-sustaining synthetic cell [42]. Most energy generating reactions are facilitated by redox processes where electron transport emerges from or drives processes like photosynthesis or phosphorylation. Enzymes, such as complex I, have been incorporated into polymersome membranes to mediate electron transfer [43]. Similarly, ATP synthase has been incorporated into polymer membranes to produce high energy phosphates like ATP [43]. In this example, the ATP synthase was driven via protons produced by bacteriorhodopsin, which was activated by light. This study illustrates how to use intermediate molecules to amplify or attenuate incoming signals. Such multi-layered signaling strategies can provide sophisticated dynamic control mechanisms over reconstituted enzyme networks.

Recently, Wilson and co-workers assembled a polymersome into the shape of a stomatocyte using a controlled shape transition [44]. In the stable bowl configuration, the stomatocyte was able to entrap platinum nanoparticles, which could in turn be used for the catalysis of reactions, such as the hydrolysis of hydrogen peroxide. Conversely, platinum particles may be used to drive the assembly of polymersomes into specific shapes of desired mobility [44]. These assemblies may be seen as a step toward constructing synthetic motile protocells.

4.2. Adhesion

Much of the work in developing vesicles has been for drug delivery [45,46]. One of the objectives of drug delivery research is to create capsules that target specific receptors, which require the addition of functional ligands to the particle surface. Peptides and single chain antibodies have been envisioned as a route to incorporate functional targeting ligands, using click chemistry and other approaches [45,47]. One of the challenges in targeting is that functionalizing the surface of surfactants is not trivial and often incomplete, leading to heterogeneous mixtures of molecules on the particle surface. Phospholipids are particularly difficult to functionalize. Some concerns regarding functionalization are ameliorated using click chemistry, which involves making polymers with terminal azide chemistry [45,48]. Also, recombinant methods would facilitate the incorporation of peptide ligands, since incorporation of peptides at the terminus of a protein would be uniform. Surprisingly, maximizing adhesion in vesicular shells is not as easy as increasing the number of functional residues on the vesicle surface, presumably because high concentrations of embedded components lead to competition and steric hinderance among functional residues; maximal adhesion usually occurs at intermediate densities of components [49,50].

Methodologies for surface functionalization are also needed for making artificial cells. The concept here is to make particles that

are the size of a cell and have the adhesive properties of cells, but might carry a payload or instruction set that allows for higher order function. For example, our laboratory has endeavored to make artificial particles that mimic the adhesive properties of leukocytes [51–53]. Leukocytes have the responsibility of binding in the microcirculation under flow. Cells like neutrophils, the first line of defense in the inflammatory response, have the responsibility of stopping, entering inflamed tissues, and releasing superoxides and other proteolyitic chemical agents. Adhesion follows a multistep cascade that involves the tethering and rolling of leukocytes, followed by firm adhesion. This multistep behavior is enabled by different membrane ligands. Leukocytes often possess two different adhesion ligands, one which performs each task, that act in collaboration to maximize adhesiveness [54]. This concept is borne out by both experiments [55] and computer simulations [56], illustrating the synergistic response of adhesion to two distinct molecules.

Using these concepts, we created artificial cells that display the adhesiveness of leukocytes, yet have the primary responsibility of delivering drugs to inflammatory sites. Early incarnations of this approach used both polystyrene microspheres [55] and poly lactic-co-glycolic acid (PLGA) microspheres that were laden with drug [57–59]. These particles were equipped with sialyl-Lewis^x, a ligand that supports rolling through binding to selectins on the endothelial cell surface, and an antibody against intercellular adhesion molecule -1 (ICAM-1), to mimic the binding of an activated integrin. The combination of the two ligands led to super-adhesion, that was beyond that achieved when either ligand acted by itself [55].

Finally we illustrated that the same adhesive motifs could be incorporated within polymersomes, leading to the construction of a leuko-polymersome. Polymersomes functionalized with sialyl Lewis^x and an antibody against ICAM-1 adhered avidly and selectively to surfaces coated with inflammatory adhesion molecules P-selectin and ICAM- I under flow. We found that maximal adhesion occurs at intermediate densities of both sialyl Lewis^x and anti-ICAM- I [53]. Leuko-polymersomes bearing these two receptor mimetics adhere under physiological shear rates to inflamed endothelium in flow chambers at a rate 7.5 times higher than to non-inflamed endothelium [53]. Later, we explored how the crosslinking of a polymersome membrane affects the dynamics of adhesion. We showed that rigidifying the membrane, by crosslinking polymers in the polymersome membrane, affects the capture, but not the rolling dynamics, of adherent leuko-polymersomes [52].

4.3. Genetic expression

A recent objective in synthetic cell research is the ability to encapsulate protein production machinery within a cell. For a recent review, please see Roodbeen and van Hest [60]. Standard demonstrations have occurred with phospholipid vesicles and green fluorescent protein (GFP). For example, POPC liposomes were used to synthesize a poly(Phe) peptide using cell-free peptide synthesis [61]. Yu and co-workers [62] produced GFP in phosphatidylcholine vesicles that were assembled from a lipid film. This method of assembly led to relatively inefficient expression [60]. Murtas and co-workers [63] improved protein expression by incorporating a minimal set of materials for protein synthesis into POPC vesicles. Additional experiments involving lipid synthesis have also been performed (see Section 10).

One dilemma in making protein expression systems is whether all of the components need to be encapsulated within the vesicle from the start, or whether additional components could be added later. Of course, cells act on the latter principle, adding material over time as necessary, but this latter approach requires controlled membrane permeability. Noireaux and Libchaber [64,65] described a method for controlling permeability by co-expressing α-hemolysin, which allows molecules up to 3000 kDa to pass into the membrane. The expression of α-hemolysin allowed the expression of eGFP for an extended period of time, compared to vesicles made in the absence of α-hemolysin [64,65].

The incorporation of components for protein synthesis in a polymer membrane was addressed recently by Weitz and co-workers, who used microfluidic methods to uniformly incorporate the synthetic machinery for MreB, a bacterial cytoskeletal protein, into polymer vesicles made from poly ethylene oxide-poly lactic acid (PEO-PLA), with additional poly lactic acid present for membrane stabilization [3]. Polymer vesicles, rather than lipid vesicles, were selected for their increased stability. A fluorescent version of Mre-B was expressed within a few hours of incorporation; see Fig. 3. The long-term goal of this group appears to be the assembly of the complete cytoskeleton of *Escherichia coli* within the polymersome membrane. To our knowledge, this is the first demonstration of the expression of a protein in the interior of a polymersome [3].

4.4. Compartmentalization

The idea to encapsulate biologically active components within vesicles has been suggested over the past 15 years. Elson and co-workers demonstrated the incorporation of functional actin within giant phospholipid vesicles [66]. Initially spherical vesicles could be forced into non-spherical shapes by actin polymerization, the degree of polymerization could be modulated by the incorporation of gelsolin, an actin nucleating molecule, and filamin, an actin filament crosslinker [66]. Actin polymerization was controlled dynamically by incorporating valinomycin in vesicles to regulate the intravesicular K^+ concentration and actin polymerization.

A variety of microfluidic strategies have emerged to encapsulate components within closed shell capsules, and these methods have evolved further to enable the encapsulation of separate compartments with distinct chemical composition and with high encapsulation efficiencies. Weitz and co-workers have developed means of co-flowing jets of different compositions to generate double emulsions that direct the assembly of membrane structures. The resulting vesicles encapsulate precise amounts of internal contents in the internal aqueous phase in the microfluidic system [67]. The composition of the internal aqueous compartment and the membrane itself can be precisely controlled using these methods. One minor difficulty with these techniques is they often involve the retention of organic solvent in the center organic phase, which must be evaporated over time to allow the assembly of a proper membrane [68]. This approach poses difficulties for the encapsulation of functional membrane proteins, as it relies on proper folding in a complex and changing environment. Polymer membranes have proven an effective alternative for constructing these membranes, as they are more robust and less prone to oxidation than their lipidic counterparts. Since it may take as long as 2 weeks for the organic solvent to be removed from the membrane [68], the stability of the surfactant used to construct it is of paramount importance. An alternative advance by Fletcher and co-workers is to push a water droplet containing appropriate composition through a supported bilayer that contains functional membranes [69]. This method can be used to control the composition and structure of the membrane, as well as the composition of the components that are held in the vesicle. Variations in pulse number, pulse voltage, and solution viscosity can be used to systematically control the vesicle size. Microspheres were co-encapsulated along with the cytoskeletal protein actin to track its polymerization into an entangled cytoskeletal network after the formation of vesicles [69].

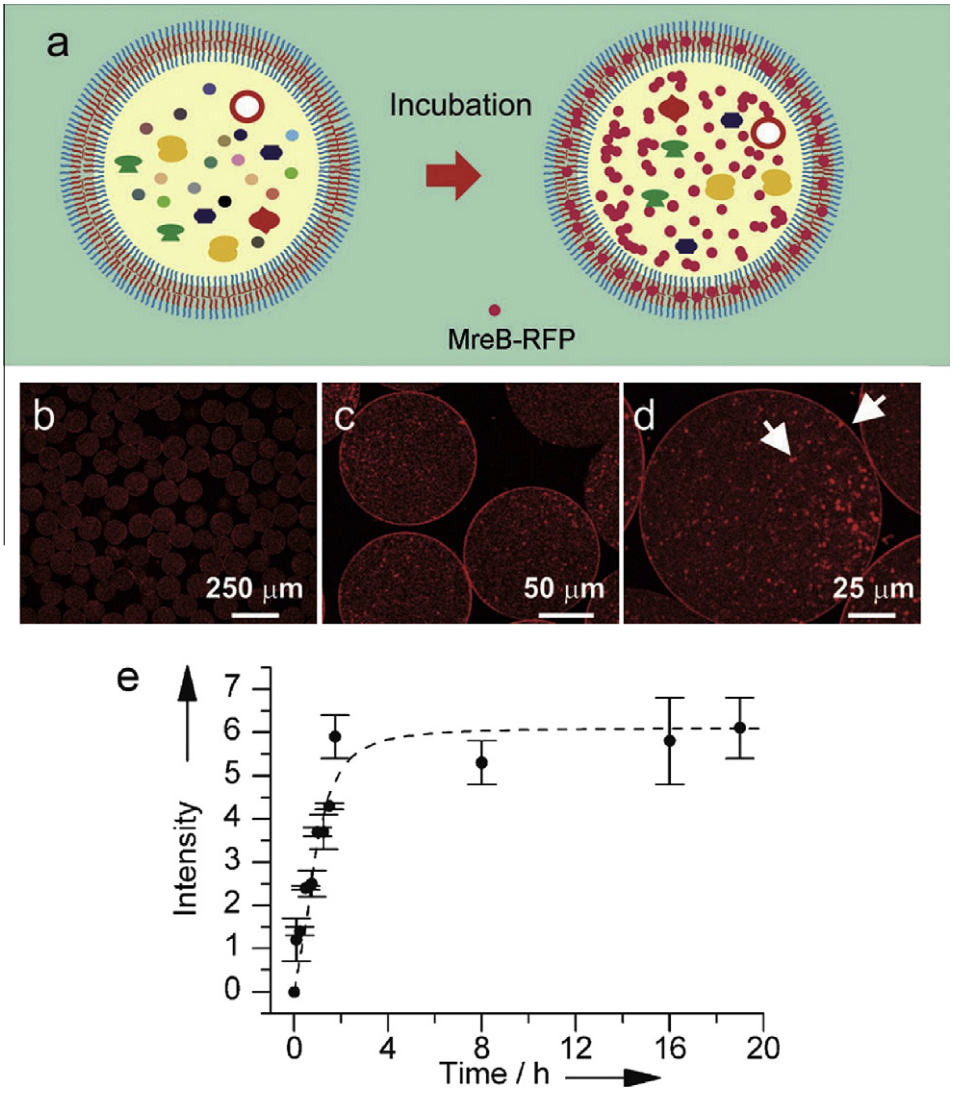

Fig. 3. MreB-RFP expression in polymersomes. (a) Schematic illustra- tion of a polymersome containing the cell-free protein expression solution. After two hours of incubation at 32 °C, the MreB-RFP protein is produced (red spots). (b–d) Confocal microscope images at different magnifications of reinforced PEG-b-PLA polymersomes after 3 h of incubation. Arrows indicate the formation of polymerized MreB-RFP patches dispersed in the inner phase and the adhesion of the protein on the membrane. (e) Fluorescence signal increases over time owing to protein expression in polymersomes. Figure and caption reproduced with permission of John Wiley & Sons from Ref. [3].

Eukaryotic cells are highly compartmentalized, with different functions being carried out in different organelles. Thus it would be advantageous to incorporate different separate compartments of distinct composition within the interior of an outer membrane. Weitz and co-workers use microfluidics to produce polymer vesicles, with encapsulated polymersomes of distinct compositions, and trigger the release of the different internal components. To accomplish this. monodisperse polymersomes were prepared from double emulsions, which in turn were injected as the innermost phase of polymersomes that were generated by microfluidics [70]. This technique could be repeated to include internal organelles of distinct compositions simultaneously. In addition, by changing the composition of each bilayer (by incorporating different amounts of distinct homopolymer), the controlled and sequential dissociation of the material from different organelles could be programmed. It is easy to imagine the extension of this encapsulation methodology to include vesicles of different composition with separate, designed tasks [70].

4.5. Communication systems

Cells are often required to migrate away from their current location or to recruit or signal to other cells. Mimicking autonomous motion with synthetic systems has become a popular means of both studying intracellular and cellular motility and engineering micro-and nano-motors that can independently display directed motion. Developing synthetic artificial capsules that possess the ability to move in response to environmental cues will be an important step towards creating an autonomous, synthetic cell.

Many groups have undertaken the study of cell motility by constructing minimal systems. Within these systems, biological molecules like cytoskeletal filaments and motor proteins have been encapsulated in order to study how dynamic assembly and disassembly of actin filaments and microtubules might generate membrane movement [71]. Actin polymerization is well understood to be vital for cell locomotion [72]. The Theriot group demonstrated that asymmetric actin polymerization can induce movement over a decade ago by creating self-propelled polystyrene beads [73].

Actin polymerization was also demonstrated within a lipid vesicle [66,74], a necessary step towards creating cytoskeletal networks within the confines of synthetic membranes.

Principles of physical chemistry have also been used to suggest directed movement in artificial systems. Within these studies, instead of polymerizing components, manipulation of Brownian motion or diffusiophoresis in asymmetric environments can be used to drive particle movement and mimic macroscopic cell motion [75,76]. Possible routes to achieve directed particle motion make use of local gradients of ions, chemical reactants, and adhesion molecules to direct particle motion [4]. In one example, electrophoretic motors have been created in which an oxidation reaction produces protons on one side of a particle and electrons on the other [77,78]. The ion flux across the particle induces its motion.

Lipid vesicles have demonstrated directed movement due to local ion gradients. Here, a negatively charged lipid vesicle was placed on positively charged supported lipid bilayers. Lipid exchange between the supported bilayer and the lipid vesicle led to the formation of a charge gradient and vesicle motion [79]. Osmotic pressure gradients, driven by a chemical reaction that generates a particle concentration gradient, can be harnessed to drive the motion of neutrally charged particles [80].

Finally, Balazs and co-workers proposed that colonies of synthetic particles could be driven to move haptotactically [81]. This theoretical work has postulated an elegant communication system between a signaling and target particle adherent to a surface. The controlled release of nanoparticles onto an adhesive surface generates a surface gradient to which a nearby targeting capsule can respond. These types of communicating capsules can potentially be reconstructed in vesicles, and would begin to mimic the type of migratory behaviors displayed by cells.

4.6. Membrane regeneration

The encapsulation of enzymes that can be used to synthesize phospholipids has obvious value for making synthetic cells, since they can be used for membrane self-replication. Kuruma and co-workers entrapped sn-glycerol-3-phosphate acyltransferase and lysophosphatidic acid acyltransferase in POPC vesicles. It was shown that both enzymes were synthesized and active in the vesicle, although their production capacity was low and no substantial remodeling of the membrane was observed [60,82].

Szostak's laboratory has been working to create self-replicating vesicles in an effort to understand how protocells, that predated our current biological cells, both functioned and may have evolved. Working primarily with simple amphiphilic molecules, like short, single chain fatty acids or oleic acid monomers, the Szostak group has demonstrated that these molecules self assemble to form highly fluid and permeable bilayer membranes, qualities that would be essential for allowing cell growth and division, and the transport of essential nutrients across, prebiotic membranes. Chen and co- co-workers demonstrated how membrane components could be transferred from one vesicle population to another as a function of differential membrane stress [83]. Vesicles containing RNA experienced increased osmotic pressure and a corresponding increase in membrane tension. Relaxed vesicles coexisting with the tensed membranes of the RNA-containing vesicles were shown to shrink as their fatty acids were transferred. The osmotically stressed and swollen vesicles, correspondingly grew upon uptake of the fatty acid chains and relaxation of membrane tension. The desorption rate of fatty acids from the relaxed vesicle population was found to increase with shorter chain length. In addition, the nature of the encapsulated osmolytes was investigated and vesicles osmotically stressed by oligomers demonstrated greater membrane exchange with relaxed vesicles than vesicles osmotically stressed by other osmolytes like tRNA or 5′UMP. The group proposed that encapsulation of RNA osmolytes and increased replication of this encapsulated material may have conferred a selective advantage for these vesicles to grow [83].

In a second approach to growing vesicle membranes, the Szostak group drove vesicle growth through incubation with fatty acid (myristoleic acid) micelles. Using this method, the authors demonstrated a cyclic model of vesicle growth and division [84]. Upon addition of fatty acid micelles, oleate vesicles grow into long, tubular vesicles shapes. When gentle shear forces were administered to the vesicle solution by air depression from a canister, the vesicles divided. Multiple cycles of growth and division were demonstrated and vesicles containing RNA were shown to continuously transfer their RNA material to daughter cells generated with each cycle of division [84].

The replication of genetic material would be an essential component in any self-replicating cell. Using highly permeable fatty acid membranes, Mansy and co-workers [84] showed how charged nucleotides could cross these membranes and enable the copying of an encapsulated DNA template. The permeability of the membranes, and thus, the ability for nucleotides and other essential biological subunits to transverse the vesicle membrane is greatly affected by the membrane composition. The increase in phospholipid content, the major lipid that makes up current biological cell membranes, decreases the permeability of vesicle membranes. Interestingly, the Szostak group has recently reported that the presence of phospholipids in oleate vesicles leads to lipid uptake from neighboring, pure component, oleate vesicles [85]. Here the decreased permeability conferred on vesicles by the addition of phospholipids accompanies an increased probability for membrane growth. Vesicles containing phospholipids may have had a selective advantage for growth, which then created a selective pressure for membranes to develop transport mechanisms to overcome the loss of permeability.

5. Concluding remarks

We are experiencing a convergence of numerous powerful technologies for the assembly of biological components into cells. Microfluidic technologies are enabling the precise reconstruction of components into synthetic cell membranes with unprecedented reproducibility and precision. The tools of molecular biology are making it possible to reconstitute the necessary machinery to make biological membranes as well as the intracellular components to generate specific molecular signals, cytoskeletal materials, or response motifs. Given advances in synthetic biology, it will soon be possible to incorporate biological components in capsules to enable a precise signaling response, for communication and dynamic response, perhaps aided by the assembly of ultra-stable membranes by novel amphiphiles that permit the long-term encapsulation and extended expression of biological components in these protocells.

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